

***Encarsia hera* Lahey & Andreason (Hymenoptera, Aphelinidae): a charismatic new parasitoid of *Aleurocybotus* Quaintance & Baker (Hemiptera, Aleyrodidae) from Florida**

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Abstract

A new, biparental species of the genus *Encarsia* Förster (Hymenoptera: Aphelinidae), *E. hera* Lahey & Andreason, **sp. nov.**, is characterized based on morphological and molecular data. The parasitoid was reared from the puparia of its host, an undescribed species of the grass-feeding aleyrodine genus *Aleurocybotus* Quaintance & Baker (Hemiptera: Aleyrodidae) collected in Gainesville, Florida. The same whitefly is newly recorded from Charleston, South Carolina, where it is a pest of ornamental Muhly grass [*Muhlenbergia capillaris* (Lam.) Trin. (Poaceae)]. A phylogenetic analysis based on a fragment of 28S ribosomal DNA in 34 *Encarsia* species placed *E. hera*, **sp. nov.**, within the *E. luteola*-group, a result concordant with its morphology. A key to the *Encarsia* species reared from *Aleurocybotus* is provided.

Keywords

DNA, new species, phylogenetics, whitefly

Introduction

Florida is home to the largest number of whitefly (Hemiptera: Aleyrodidae) species in the United States (Hodges and Evans 2005; L. Deeter, pers. comm.). While many of these species have been described, the discovery of new aleyrodid species is not uncommon, nor is the establishment of extralimital, adventive whiteflies [e.g., *Aleurodicus rugioperculatus* Martin; *Asiothrixus antidesmae* (Takahashi); *Singhiella simplex* (Singh)] (Stocks 2013). It has been known for several years that an undescribed species of the genus *Aleurocybotus* Quaintance & Baker exists in Florida (G. Evans, pers. comm.). The undescribed species can be separated from its congeners by a combination of morphological characters found in the immature and adult stage (G. Evans and N. von Ellenrieder, pers. comm.).

Species of *Aleurocybotus* are unusual among whiteflies in that they feed exclusively on grasses (Poaceae) and sedges (Cyperaceae) (Russell 2000). Among four known species, *A. cereus* Martin was described from Belize (Martin 2005). The remaining three described species are recorded from the west and east coasts of the United States (Quaintance 1899; Russell 1964; von Ellenrieder and Bailey 2022), and two of these, *A. graminicolus* (Quaintance) and *A. occiduus* Russell, have been collected in Florida.

Natural enemies associated with *Aleurocybotus* include several species of chalcidoid parasitoid Hymenoptera in four genera: *Encarsia* Förster and *Eretmocerus* Haldeman (Aphelinidae) (Myartseva et al. 2009); *Metaphycus* Mercet (Encyrtidae) (Myartseva and Cancino 2010); and *Euderomphale* Girault (Eulophidae) (LaSalle and Schauff 1994). The latter three genera are infrequently collected from *Aleurocybotus*; for discussions of those species, the reader is referred to the above references.

The genus *Encarsia* contains approximately 450 described species (Kresslein et al. 2020), although conservative estimates of the number of species is 10× that number (Polaszek et al. 2009). The primary hosts of *Encarsia* are whiteflies, although other groups of sternorrhynchous Hemiptera and insect eggs may also be attacked (Polaszek 1991; Evans et al. 1995; Heraty et al. 2008; Polaszek and Luft Albarracin 2011). Several species have been at the center of successful biological control programs, which makes this genus of considerable economic importance (Clausen and Berry 1932; Hart et al. 1978; Sailer et al. 1984; Hoddle et al. 1998). The purpose of this study is to describe a new species of *Encarsia* reared from a pestiferous, undescribed species of *Aleurocybotus* from the southeastern United States, place the *Encarsia* species within the context of the genus based on a phylogenetic analysis of 28S ribosomal DNA, and provide a key to the species of *Encarsia* known to attack *Aleurocybotus*.

Materials and methods

Specimen collection

Immature and adult *Aleurocybotus* were collected in 2022 from infested ornamental Muhly grass [*Muhlenbergia capillaris* (Lam.) Trin. (Poales: Poaceae)] in Gainesville, Florida (Alachua County) and Charleston, South Carolina (Charleston County), USA.

Representative series of unparasitized puparia and other whitefly life stages were collected into 95% ethanol and slide-mounted following the protocol of Martin (2004), except that clearing of specimens took place in an ATL lysis buffer-Proteinase K solution, the first step in a non-destructive DNA extraction protocol that retains the cuticle of the insect for subsequent morphological examination. Parasitoid specimens were reared directly from their host. Parasitized whitefly puparia were excised from portions of leaf tissue using a cork borer (5 mm diameter), placed in size 0 gelatin capsules (Pure Planet Products, Scottsdale, Arizona), and monitored daily for emergence. Reared parasitoid specimens were killed directly in 95% ethanol and stored at -20 °C until DNA extraction.

DNA Extraction & PCR

Genomic DNA was extracted from *Aleurocybotus* puparia, adults, and single wasps using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Individual specimens were first removed from ethanol and allowed to air dry on a Kimwipe for approximately 30 s. The specimen was then transferred to an Eppendorf tube containing 90 µl ATL lysis buffer to which 20 µl proteinase K was added. Tissue digestion was achieved by incubating the reaction mixture for 8 hrs at 56 °C. Tubes were agitated periodically, by hand, to ensure the specimen remained in the reaction mixture at the bottom of the tube. Most specimens were satisfactorily cleared after 8 hrs in the reaction mixture, but some were left for 24 hrs without damaging the specimen. Following tissue digestion, specimens were removed from the reaction mixture directly into distilled water where they remained until slide-mounting. Extracted parasitoids were placed on microscope slides following the protocol of Polaszek et al. (2013). The extract was then processed per the manufacturer's instructions except that the AE buffer used in the final elution step was warmed to 55 °C, and the final elution volume per sample was 50 µl. DNA extracts were then stored at -20 °C until use in PCR.

The standard 5' barcode region of the cytochrome c oxidase subunit I (COI) gene (Folmer et al. 1994) and the 28S D2 and D3 domains of the 28S large ribosomal subunit (28S-D2-3) were targeted for amplification by PCR. The standard barcode primers (HCO2198/LCO1490) do not adequately amplify this region from many chalcidoids. Therefore, we utilized the two primer sets from Fusu and Polaszek (2017) that produce overlapping 'mini-barcode' sequences that can be assembled into a full consensus barcode. Primer sequences for each genomic region are listed in Table 1. The thermocycling conditions for the 28S-D2-3 region followed Andreason et al. (2019) and that of COI followed Fusu and Polaszek (2017) and Polaszek et al. (2022). PCR amplicons were visualized on a 1.5% agarose gel in 1X TAE buffer stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA) and a TrackIT 1 Kb Plus DNA Ladder (Invitrogen, Waltham, MA, USA) to estimate product sizes. Both strands of each amplicon were sequenced on an ABI 3730xl DNA Analyzer by Eton Bioscience, Inc. (Research Triangle Park, NC, USA). Forward and reverse sequences of each amplicon were assembled with the Geneious assembler at the Highest Sensitivity/Slow setting in Geneious Prime (version 2022.0.2). Newly generated sequences for all *Encarsia* species used in the phylogenetic analyses have been deposited in GenBank (Table 2).

Table 1. PCR primer sets used in this study.

Primer	Orientation	Region	Sequence (5'–3')	Reference
MChaF1	Forward	COI A	CCTCGAATAAATAATATAAGATT	Fusu and Polaszek 2017
HCO2198	Reverse		TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
LCO1490M	Forward	COI B	CAACAAATCATAAAGATATTGG	Fusu and Polaszek 2017; Folmer et al. 1994
MChaR1	Reverse	28S-D2-3	CCYGTTCCTCAAYAAATATTCT	Fusu and Polaszek 2017
D23F	Forward		GAGAGTTCAAGAGTACGTG	Park and O’Foighil 2000
D3B	Reverse		TCGGAAGGAACCAGCTACTA	Nunn et al. 1996; Whiting et al. 1997

Table 2. CUIDs, host records, GenBank accession numbers, and sequence lengths associated with the *Encarsia* specimens newly sequenced for this study.

Taxon	CUID	Host	GenBank Accession	Sequence Length (bp)
<i>Encarsia citrella</i>	OSUC 835434	<i>Aleuroplatus</i> sp.	OP133209 (28S)	901
<i>Encarsia protransvena</i>	OSUC 835443	<i>Parabemisia myricae</i>	OP133210 (28S)	742
<i>Encarsia</i> sp.	OSUC 835445	<i>Tetraleurodes</i> sp.	OP133211 (28S)	746
	OSUC 835446		OP133212 (28S)	745
<i>Encarsia lahorensis</i>	OSUC 835456	<i>Dialeurodes citri</i>	OP133213 (28S)	1,085
<i>Encarsia</i> sp. ^a	OSUC 863826	Unknown	OP133214 (28S)	713
<i>Encarsia hera</i>	OSUC 863846	<i>Aleurocybotus</i> sp.	OP146609 (28S)	494
			OP270223 (COI)	673
			OP133215 (28S)	750
	OSUC 863847		OP270224 (COI)	674

^a Specimen was collected with a sweep net.

Phylogenetic analyses

Phylogenetic analyses were conducted following Polaszek et al. (2021). Maximum likelihood phylogenies were estimated for the 28S-D2-3 region of 34 *Encarsia* species using IQ-TREE (v. 2.1.3) (Minh et al. 2020). This gene region in *Encarsia* is fast-evolving and accumulates mutations at a rate that provides sufficient phylogenetic signal to delimit species and species-groups with minimal noise (Heraty 2004; Polaszek et al. 2009). Sequences were aligned with MAFFT (v. 7.429) (Katoh et al. 2013) using the E-INS-i algorithm. The best nucleotide substitution model was selected with ModelFinder (Kalyaanamoorthy et al. 2017), and branch support was estimated by 1000 ultrafast bootstrap replicates with the –bnni flag enabled to reduce the negative impact of model violations (Hoang et al. 2018). We performed 25 independent tree searches and present the tree with the best (greatest) log-likelihood score. Two coccophagine aphelinids were selected as outgroups: *Coccophagoides fuscipennis* (Girault) (GenBank: AF254248.1) and *Pteroptrix chinensis* (Howard) (GenBank: KF778628.1).

Databasing

The specimens listed in the Material Examined section of the species description have been accessioned in The Ohio State University’s Museum of Biological Diversity data-

base (<https://mbd-db.osu.edu/>). The numbers prefixed with “OSUC” are unique identifiers for the individual specimens. Details of the data associated with these specimens may be accessed at the above URL by entering the unique specimen identifier (e.g., OSUC 863846) in the form (note the blank space after the acronym).

Morphology

Morphological terminology follows Schmidt and Polaszek (2007). Relative lengths of morphological features were taken from the slide-mounted holotype and paratype as depicted in Heraty and Polaszek (2000).

Imaging

Slide-mounted specimens were imaged with a Keyence BZ-X810. Photographs of card-mounted insects were captured using a Macroscopic Solutions Macropod Micro Kit, with optical slices rendered in Helicon Focus. Composite images from each imaging system were imported into Adobe Photoshop 2022 to correct for brightness and contrast.

Collections

The slide-mounted holotype (OSUC 863846) and paratype (OSUC 863847) of *E. hera*, sp. nov., are deposited in the Smithsonian National Museum of Natural History (USNM), Washington, DC, USA. The two card-mounted paratypes (OSUC 863886; OSUC 863887) are deposited in the Florida State Collection of Arthropods (FSCA), Gainesville, Florida, USA.

Results

Taxonomy

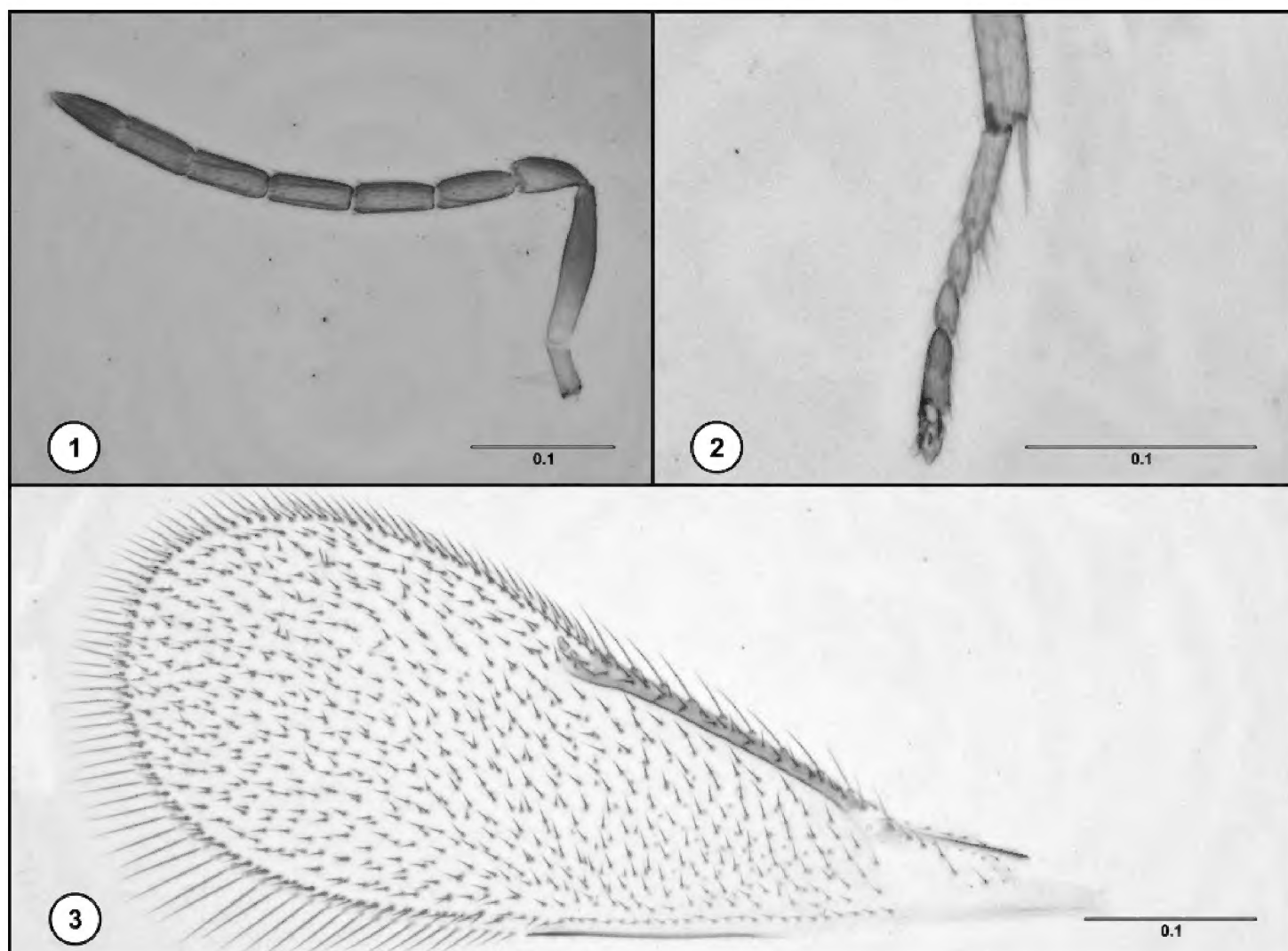
Encarsia hera Lahey & Andreason, sp. nov.

<https://zoobank.org/3C75DFAF-5FFF-44A1-947F-E34DF7F9DF3C>

Figs 1–5

Species-group placement. *Encarsia luteola*-group.

Diagnosis. *Encarsia hera*, sp. nov., can be differentiated from other members of the *E. luteola*-group by outstanding coloration of the mesoscutellum and metasoma. Most *E. luteola*-group species have a concolorous mesoscutellum and a predominately yellow metasoma. *Encarsia hera*, sp. nov., differs from these species by the paired brown patches in the posterior half of the mesoscutellum and the predominantly brown metasoma of the female. *Encarsia guadeloupae* Viggiani also has a dark metasoma; however, in that species T1 is completely dark and the clava is 2-merous, whereas the lateral portions of T1 are yellow and the clava is 3-merous in *E. hera*, sp. nov.



Figures 1–3. *Encarsia hera* Lahey & Andreason, female holotype (OSUC 863846) **1** Antenna, lateral view **2** Mesotarsus, dorsal view **3** Fore wing, lateral view. Scale bars in millimeters.

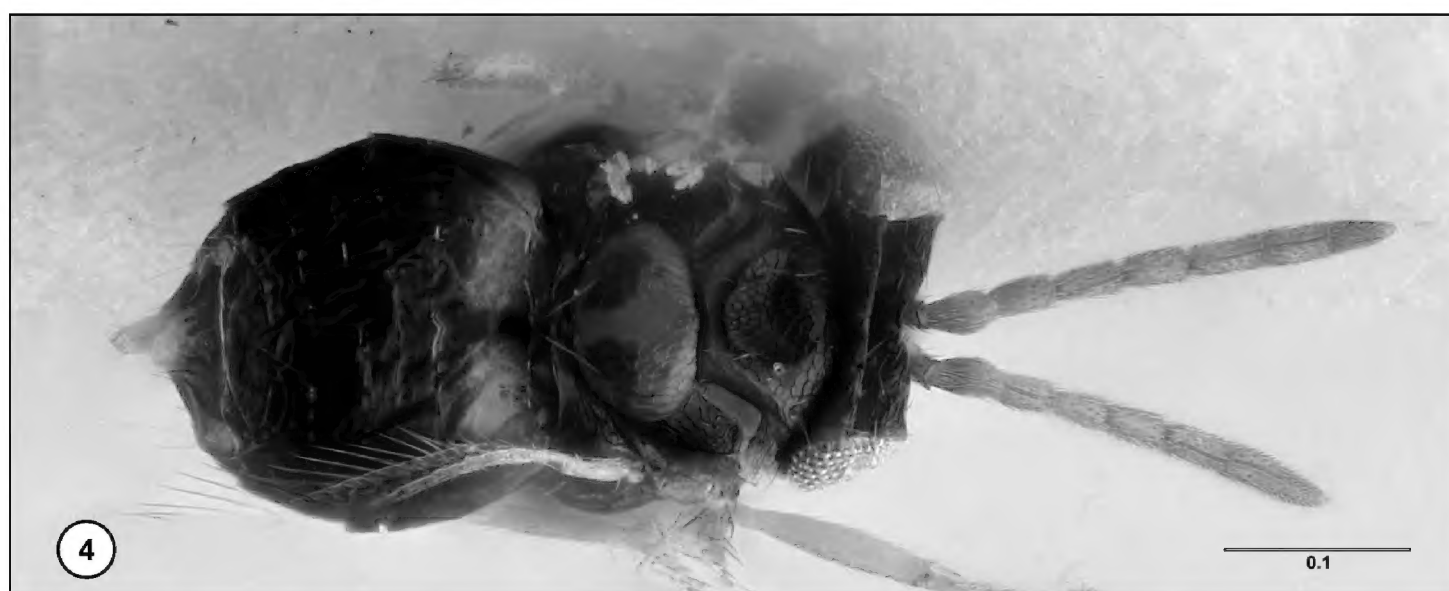


Figure 4. *Encarsia hera* Lahey & Andreason, female paratype (OSUC 863886), habitus, dorsal view. Scale bar in millimeters.

Description (female). Coloration. Body: predominately dark brown. Head: dark brown, except for pale areas on frons adjacent to compound eyes and a transverse strip on vertex anterior to ocellar bars. Antenna: yellow, except for fuscous apical clavomere (F6). Mesosoma: dark brown, except for yellow lateral and posteromedial margin of

mesoscutum, mesoscutal side lobe, anterodorsal portion of acropleuron, and most of mesoscutellum. Mesoscutellum: predominately yellow with two conspicuous brown spots in posterolateral half. Fore and hind wings: hyaline, venation fuscous. Legs: pale yellow, except for fuscous apical tarsomere (tarsomeres 4 + 5 fused) on midleg and apical three tarsomeres on hindleg. Metasoma: dark brown, except for lateral portions of T1 which appear transparent/opalescent. Ovipositor: third valvulae yellow.

Head. Antennal formula: 1-1-3-3. Length of pedicel relative to F1: 0.8. Length of F1 relative to F2: 0.9. Length of F2 relative to F3: approximately equal. Number of multiporous plate sensilla on F1–F6: 1-2-2-3-3-3. Sculpture of stemmaticum: aciculate. Sculpture of frons ventral to transfacial line: indiscernible. Sculpture of frons dorsal to transfacial line: transversely imbricate.

Mesosoma. Number of setae on midlobe of mesoscutum: 16. Number of setae on side lobe of mesoscutum: 2. Number of setae on axilla: 1. Proximity of campaniform sensilla on mesoscutellum: ≥ 5 sensillar widths apart. Distance between anterior pair of mesoscutellar setae: equal to distance between posterior pair of mesoscutellar setae. Length of mesoscutellar setae: anterior pair distinctly shorter than posterior pair. Tarsal formula: 5-4-5. Length of midtibial spur: $0.8\times$ length of midbasitarsus.

Metasoma. Number of paired setae on T1–T6: 0-1-2-1-3-3. Length of ovipositor: $0.8\times$ length of midtibia. Apical portion of 3rd valvulae: chisel-tipped, inner margin longer than outer margin. Length of 3rd valvulae relative to 2nd valvifer: $0.7\times$.

Wings. Length of fore wing: $2.7\times$ width. Asetose area below stigma vein: absent. Length of marginal fringe: $0.3\times$ maximum width of disc. Number of setae in basal cell region: 5. Arrangement of setae in basal cell: linear, originating and forming a 45° angle with submarginal vein. Number of setae on submarginal vein: 2. Number of setae along anterior of marginal vein: 8; 9.

Description (male). **Coloration.** Same as female, except for the darker mesoscutellum and T1 is dark throughout.

Morphology. Sculptural patterns very similar to female. Mesoscutellar sculpture: weak medially, large reticulations laterally. Number of antennomeres: 8. Condition of F6: articulate with F5, not fused or partially fused.

Distribution. Florida (USA).

Host. *Aleurocybotus* sp. nr. *cereus* (Hemiptera: Aleyrodidae).

Etymology. Named for the Hera of Greek mythology, one of the Twelve Olympians, Queen of the Gods, and protector of women from harm during childbirth.

Material Examined. **Holotype**, female: **USA:** Florida, Gainesville, $29^\circ36'3''\text{N}$, $82^\circ25'13''\text{W}$, 19.vi.2022, ex. *Aleurocybotus* n. sp. on ornamental Muhly grass (*Muhlenbergia capillaris*), Z. Lahey, OSUC 863846 (deposited in USNM). **Paratypes:** **USA:** collection data identical to holotype, 1 female, 2 males, OSUC 863847 (USNM); OSUC 863886, 863887 (FSCA).

Phylogenetic analyses. The alignment of the 28S-D2-3 region in the 36 taxa was 1,037 characters long (base pairs plus gaps) and the model of nucleotide evolution was SYM+I+G4. In all analyses, the *E. luteola*-group was recovered as monophyletic with maximum ultrafast bootstrap support (UFBS = 100; Fig. 9). *Encarsia hera*, sp. nov., was nested within the *E. luteola*-group, as the sister taxon to *E. formosa* Gahan (UFBS = 99; Fig. 9), and *E. luteola*



Figure 5. *Encarsia hera* Lahey & Andreason, male paratype (OSUC 863887), habitus, dorsolateral view. Scale bar in millimeters.

Howard was recovered as the sister taxon to *E. hera* sp. nov. + *E. formosa* (UFBS = 95; Fig. 9). An expanded analysis of the same gene region with additional *Encarsia* species recovered the same sister group relationships between *E. luteola*, *E. hera* sp. nov., and *E. formosa* as those in Fig. 9 (Suppl. material 1), as did a trimmed version (495 characters) of the original dataset (Suppl. material 2). While this article was in press, we were alerted that two taxa (three sequences) used in the phylogenetic analyses are misidentified in GenBank. The sequences corresponding to accessions AF223366.1 and AF223367.1 belong to *E. californica* Polaszek and AY360217.1 corresponds to *E. dispersa* Polaszek. Both *E. meritoria* Gahan and *E. haitiensis* Dozier have never been sequenced (A. Polaszek, pers. comm.).

Comments. Members of the *E. luteola*-group are recognized by having 4 mesotarsal segments and a fully setose wing disc (Gahan 1924; Polaszek et al. 1992). This species group has been recovered as monophyletic in several phylogenetic analyses of 28S rDNA (Babcock et al. 2000; Schmidt et al. 2006), although no analysis has yet to include all described species.

The sister group relationship between *E. formosa* and *E. hera*, sp. nov., recovered in our study breaks the longstanding paradigm that *E. formosa* and *E. luteola* are likely each other's most closely related living relative (Babcock and Heraty 2000). This is an

- 2 Mesoscutellum with two brown markings in posterior half (Fig. 4); clava 3-merous (Fig. 1) ***Encarsia hera* Lahey & Andreason, sp. nov.**
- Mesoscutellum concolorous throughout (Fig. 6); clava 2-merous (Fig. 7) ... **3**
- 3 Dorsolateral surface of metasoma light yellow, concolorous with remainder of metasoma ***Encarsia luteola* Howard**
- Dorsolateral surface of metasoma brown, contrasting with remainder of metasoma (Fig. 8) ***Encarsia longitarsis* Myartseva**

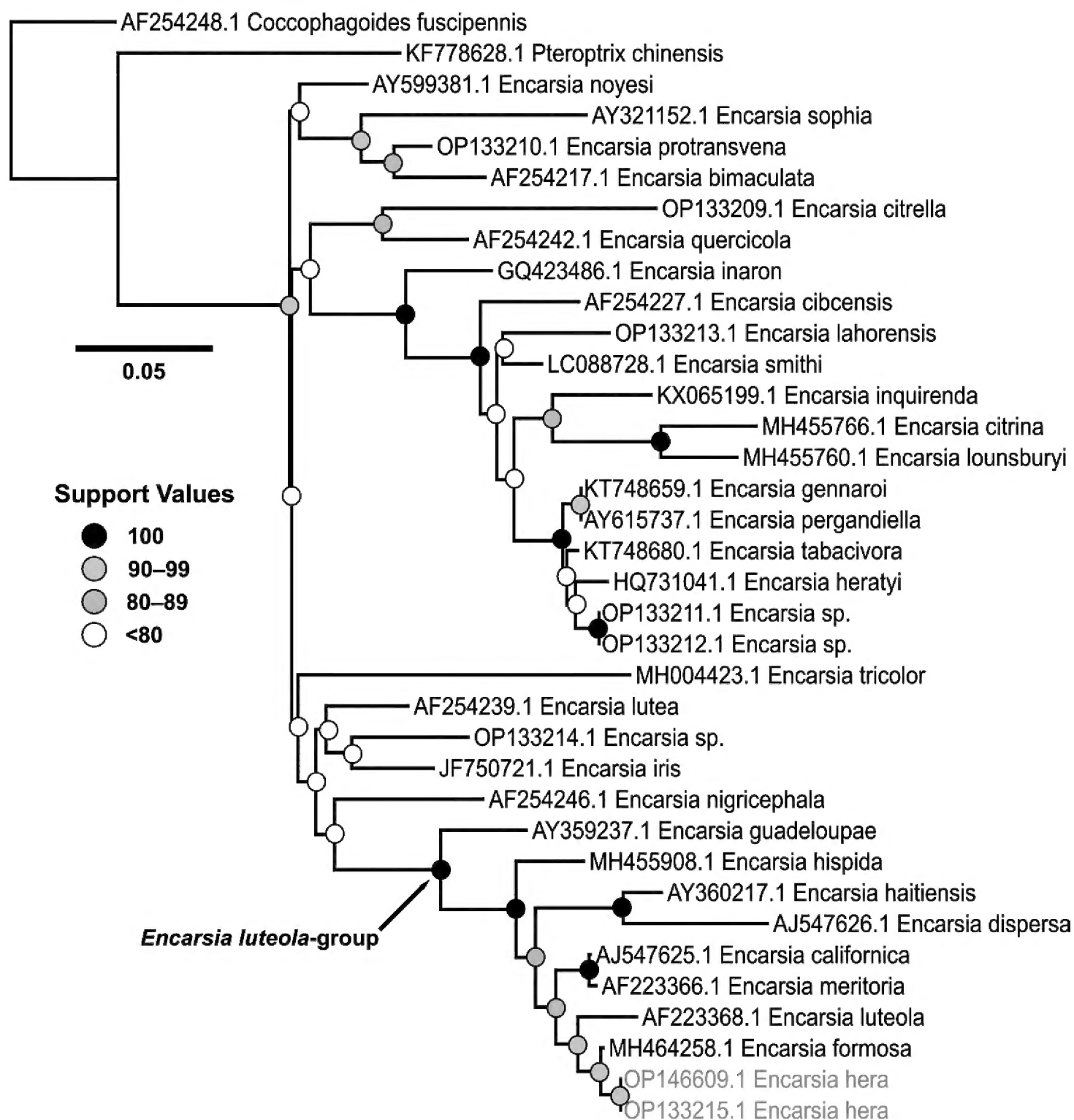


Figure 9. Maximum likelihood phylogeny of the 28S-D2-3 region in 34 *Encarsia* and two outgroup species. The number under the scale bar indicates the number of expected nucleotide substitutions per site. Ultrafast bootstrap supports values are indicated by colored circles at nodes. GenBank accession numbers beginning with OP correspond to specimens newly sequenced for this study.

Discussion

The arthropod fauna of Florida is in a constant state of flux, notably because of its position as a botanical import hub of the countries that comprise the Caribbean, South America, Europe, and Asia (Stocks 2013). Each of these regions harbors exotic whitefly species with the potential to become invasive and effect economic losses in the United States. The provenance of the new species of *Aleurocybotus*, first found in Florida, and now found in South Carolina, as well as its parasitoid, is uncertain. The earliest collections of this whitefly are from January 1988 in Wabasso, Florida, on Muhly grass (L. Deeter, pers. comm.). Muhly grass is a common perennial ornamental that is native to eastern North America and is the only reported host of this whitefly species.

The evidence for the undescribed whitefly as adventive is based on a comparison of its morphology with other described species of the genus. Puparia of the new *Aleurocybotus* secrete copious amounts of flocculent wax, a characteristic exhibited by both *A. cereus* and *A. mojaviensis* von Ellenrieder & Bailey, species from Belize and California, respectively (Martin 2005; von Ellenrieder and Bailey 2022). These species share a glandular zone on the dorsal submedian area of the nymphal stages that is not present in either named species known to occur in Florida (von Ellenrieder and Bailey 2022).

Surveys of parasitoid Hymenoptera primarily associated with *Bemisia* Quaintance & Baker in Florida, the Caribbean, and Latin America failed to recover *E. hera*, sp. nov., from that host (Stansly et al. 1997; Schuster et al. 1998). Lahey (2014) built upon these efforts by expanding the taxon sampling to additional whitefly genera in Florida. *Encarsia hera*, sp. nov., was not reared from the 13 non-*Bemisia* genera collected over a 6-year period, but these collections did not include *Aleurocybotus* (Lahey 2014; Stocks 2016; Lahey and Polaszek 2017). Given that this parasitoid has not been reared from any other whitefly genus in Florida, it apparently established with its host on infested plant material and has persisted since the original introduction, sometime before 1988. Alternatively, *E. hera*, sp. nov., may be a recent introduction or have expanded its host range to include *Aleurocybotus*. Testing either hypothesis will require continued sampling of whiteflies and their parasitoids from a broad geographic range.

The discovery of a new *Encarsia* species is not surprising. *Encarsia* is the most speciose genus in the family Aphelinidae and has been labeled ‘megadiverse’ based on a suite of morphological, genetic, and biological characteristics that account for the estimated 4,000 plus species thought to exist in nature (Polaszek et al. 2009). This estimate may even be considered conservative in that it does not consider the lack of knowledge regarding the biodiversity of their hosts, mostly whiteflies (approx. 1,600 described species; Martin and Mound 2007) and armored scale insects (2,700 described species; García Morales et al. 2016), new species of which are frequently uncovered. Based on these numbers and that Florida is home to the most diverse assemblage of whiteflies in the United States, we expect the number of new *Encarsia* and whiteflies to grow as scouting for these economically important pests, and their natural enemies, continues.

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Supplementary material 1

Maximum likelihood cladogram of the 28S-D2-3 region in 71 *Encarsia* and two outgroup species (1,070 sites, SYM+R3)

Authors: Zachary Lahey, Alvin M. Simmons, Sharon A. Andreason

Data type: image (svg file)

Explanation note: Maximum likelihood cladogram of the 28S-D2-3 region in 71 *Encarsia* and two outgroup species (1,070 sites, SYM+R3). Ultrafast bootstrap supports values are indicated on branches. GenBank accession numbers beginning with OP correspond to specimens newly sequenced for this study. Branches in red correspond to members of the *E. luteola*-group.

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Link: <https://doi.org/10.3897/jhr.94.94677.suppl1>

Supplementary material 2

Maximum likelihood cladogram of a trimmed version of the 28S-D2-3 dataset analyzed in Fig. 9 (495 sites, GTR+F+G4)

Authors: Zachary Lahey, Alvin M. Simmons, Sharon A. Andreason

Data type: image (svg file)

Explanation note: Maximum likelihood cladogram of a trimmed version of the 28S-D2-3 dataset analyzed in Fig. 9 (495 sites, GTR+F+G4). Ultrafast bootstrap supports values are indicated on branches. GenBank accession numbers beginning with OP correspond to specimens newly sequenced for this study. Branches in red correspond to members of the *E. luteola*-group.

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